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Amy Yost

Amy Yost
Date of Signature 10/7/2005

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Dace et al.

Group Art Unit: 1639

Serial No.: 09/879,279

Examiner: Epperson, Jon D.

Filed: June 12, 2001

Docket No.: 1392/18/2

Confirmation No.: 3524

For: IN VITRO CAPTURE OF NUCLEIC ACIDS VIA MODIFIED OLIGONUCLEOTIDES AND MAGNETIC BEADS

****

DECLARATION PURSUANT TO 37 C.F.R. § 1.131

Commissioner for Patents
Washington, D.C. 20231

Sir:

1. We, Gayle Dace and William Kimmerly, are the co-inventors of the invention disclosed and claimed in the subject above-captioned U.S. Patent Application Serial No. 09/879,279.
2. We have had the opportunity to review the Official Action mailed April 7, 2005 from the U.S. Patent and Trademark Office for the above-referenced U.S. patent application.
3. We have also reviewed the following document cited by the United States Patent and Trademark Office in the Official Action mailed on July 13, 2004:

(a) U.S. Published Patent Application No. 2003/0077609 A1 (the '609 application).

4. The subject matter embodied in claims 1-8, 11-15, 18-24 and 31 of the subject U.S. Patent Application Serial No. 09/879,279 was invented prior to the earliest claimed priority date of March 25, 2001 of the '609 and '598 applications. The inventive activity occurred in the United States.

5. Attached hereto as **Exhibit A** is a true and accurate copy of consecutively numbered laboratory notebook pages documenting experiments performed involving the subject matter embodied in the pending claims. Exhibit A provides evidence of the subject matter recited in the pending claims and predates the earliest claimed priority date of March 25, 2001 of the '609 and '598 applications.

6. The first page of Exhibit A is a reproduction of p. 146 of a notebook entry entitled "New LNA Oligos" and records experimental conditions related to capturing specific target nucleotide sequences utilizing locked nucleic acids (LNAs), including providing nucleotide sequences of exemplary LNAs and characterizations of the LNAs and reagent quantities and conditions used in the experiments.

7. Specifically, p. 146 describes one or more modified oligonucleotide conjugates comprising at least one LNA and a linking molecule. The modified oligonucleotide referred to as "Torrey-2" comprises the listed sequence of $(GC)_6$, with the LNA residues in bold. The linking molecule used for "Torrey-2" was biotin.

8. The second page of Exhibit A is a reproduction of p. 147 of a notebook entry entitled "LNA Capture That Worked" and records further experimental conditions and reagents related to capturing specific target nucleotide sequences utilizing LNAs and experimental results.

9. The specific experimental conditions labeled "CN" and notated with a box pointing to plate T2NC01 are labeled as "WORKED" on the notebook entry of

p. 147. This comment is indicative of the successful capture of specific target nucleotide sequences utilizing an LNA.

10. Page 147 of the notebook describes incubating a sample of nucleic acids with the LNA conjugate to thereby form one or more hybridized duplexes, wherein each duplex comprises a target simple sequence repeat ("SSR") portion and an LNA conjugate. The boxed data indicates that 2.5µl of tomato library DNA was incubated with Torrey-2 LNA conjugate and Buffer C to form a hybridized duplex.

11. The third page of Exhibit A is a reproduction of p. 150 of a notebook entry entitled "LNA Capture Protocol" and describes the protocol used to acquire the data set forth on pages 146 and 147, wherein the hybridized duplexes were contacted with a linking source so that the linking molecule formed a bond with the linking source to capture the LNAs. Specifically, the protocol recites that the biotinylated LNAs were contacted with streptavidin-coated magnetic beads and incubated to allow the biotin to form a bond with the streptavidin-coated beads.

12. The protocol on p. 150 recites that the hybridized duplexes were then separated from the sample of nucleic acids by extracting the linking source from the sample, followed by a washing step and an incubation step so that the target SSRs dissociate from the LNA conjugates and the magnetic beads. Specifically, the magnetic beads were separated from the sample of nucleic acids by use of a magnet, the beads washed eight times in Buffer C, and incubated in Buffer E in order to separate the SSRs from the beads. This step is also recited on the notebook entry of p. 147: "90°C in 150µl Buffer E – 20 min.".

13. The protocol on p.150 recites that the SSRs are ethanol precipitated overnight, and purified using a PCR purification kit.

14. The protocol on p.150 recites that DH12S cells were transformed with the SSR DNA, grown overnight, the colonies picked, and stored at a temperature of -80°C for sequencing.

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15. **Exhibit B** presents the results of sequence data chromatographs establishing that the SSRs were recovered and that the experiment was successful. Specifically, well E04 of plate T2NC01 was sequenced and an SSR $(CA)_6$ was discovered at bases 221-232 which is shown underlined. Further, well F09 of plate T2NC01 was sequenced and an SSR $(CA)_8$ was discovered at bases 131-142 which is shown underlined.

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 9-28-05 By: Gayle Dace, M.S.

Gayle Dace, M.S.

Date: _____ By: _____

William Kimmerly

[redacted]

Project No.
Book No.
TITLE new LNA oligos

46

om Page No.

Sample ID	Net Abs 230.0nm	Net Abs 260.0nm	Net Abs 280.0nm	260.0/ 230.0	260.0/ 280.0	Dil Fact.	Conc.
TORREY1	0.1416	0.4132	0.2326	2.91867	1.77598	1.0000	413.1621
2	0.1436	0.4207	0.2366	2.93037	1.77819	1.0000	420.7299
TORREY2	0.0381	0.1470	0.0560	3.86056	2.62547	1.0000	146.9856
4	0.0457	0.1498	0.0586	3.27741	2.55746	1.0000	149.8367

IDO Number (Oligo ID)	Sequence	ODs (260 nm)	Anion Exchange Chromatography Results	Other Information
3985 Torrey-1	5'-BIOTIN-dGTT dGTT dGTT dGTT -3'	11.5	94.1 % a2+(4-64%base	56°C - 85°C
3986 Torrey-2	5'-BIOTIN-GTG TGT GTG TGT-3'	3.5	96.1 % oligo 5 T_m + 3-8%base 36	72°C - 80°C

1 ug non-linear tomato DNA / 1 ug linear tomato library DNA
1.8ul 265ng Oligo Torrey 2 / 1.8ul 265ng Oligo 2 (Torrey)
Buffer F / Buffer F
80°C / 45 min

1 ug non-linear tomato lib / 1 ug linear tomato library
75ng oligo 2 / 75ng Torrey 2
Buffer C / Buffer C

To Page N

Witnessed & Understood by me,
[redacted]
Date
[redacted]

Invented by [redacted]
Recorded by
Date
[redacted]

[redacted] [redacted] [redacted]

Project No.

LNA capture thal worked — Book No. — 147

ge No.

EM

2.5 µl non-lin Tom library
20°Y 1.8 µl Torrey 2 LNA →
45.7 µl Buffer F →
50 µl
for clonis

FL

3 µl Tom library
1.8 µl
45.2 µl
50 µl
no clonis

[redacted]

CN

2.5 µl non-lin tomato lib
76 mT → 2.6 µl Torrey 2 LNA (1:5 dil) →
54.9 Buffer C →
60 µl

CL

3 µl lin library
→ 2.6 µl
54.4 µl
60 µl no clones

80°C for 30 min

from vehicle

90°C in 150 µl Buffer E - 20 min
bring up to 350 µl

WORKED

EtOH ppt (with 2 vol H2O)
bring up in 100 µl TE

purify with PCR kit

T2NCO1 plate

complete protocol on p 150

HT

To Page No.

sed & Understood by me,

Date [redacted]

Invented by Gayle Dea

Recorded by

Date [redacted]

BEST AVAILABLE COPY

Project No._____
Book No._____ TITLE LNA Capture Protocol used on p 14

From Page No. 147

LNA Capture Protocol

Hybridization Buffer C: 100mM NaCl
10 mM Na_2PO_4 (pH 7.0)

Dissociation Buffer E. 1.0 M Tris-Cl (pH 9.0)
0.5 mM EDTA

Genomic library in pBluescript KS-/sacB construct.

Hybridization reaction:

1 ug library
75 ng 5'-biotinylated LNA oligo $(GT)_6$ (Proligo LLC; Boulder, CO)
Buffer C to 60 ul

Incubate at 80°C for 50 min (temperature specific to oligo sequence).

Add 60 ul of washed Dynal M-280 streptavidin-coated magnetic beads and incubate in water bath at 50°C for 80 min, shaking every 10 min to maintain suspension (could use shaker in a 50°C incubator).

Very briefly spin to move all liquid to bottom of tube. Separate beads by use of Dynal magnet. Remove liquid and wash beads 8 times with 500 ul of Buffer C.

After final wash, add 150 ul Buffer E and mix. Incubate at 90°C for 20 min (temperature specific to oligo sequence).

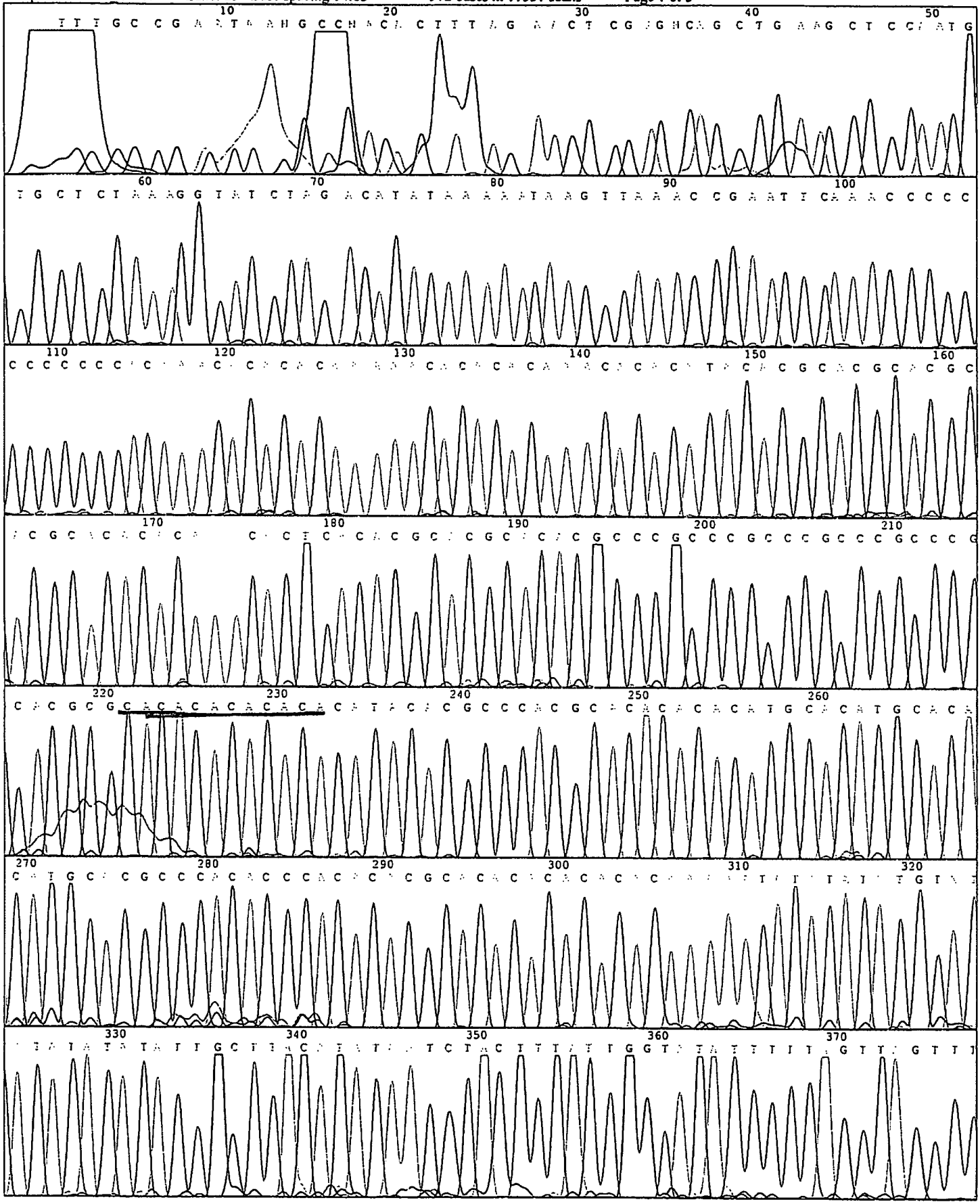
Remove and save liquid; discard beads. Add 200 ul of ddH_2O (pH 5.0), 35 ul of 3 M NaOAc (pH 5.2), and 875 ul of 100% EtOH. Precipitate at -20°C overnight. Spin in cold at 14,000 rpm for 30 min Wash with 500 ul ice cold 70% EtOH and spin for 20 min. Dry and add 100 ul 1X TE (pH 8.0). Purify using Qiagen PCR purification kit.

Transform Life Technologies DH12S electrocompetent cells with 1 ul DNA to 40 ul cells. Using the BioRad Gene Pulser II, the settings are 25 uF, 200 Ω, and 1.8 kV.
Add 960 ul of SOC broth, and gently shake for 1 hour at 37°C. Plate 75 ul of transformation on LB amp plates containing 5% sucrose. Grow overnight and pick colonies into LB w/8% glycerol. Grow overnight, place in -80°C for sequencing.

To Page N

Witnessed & Understood by me.	Date	Invented by	Date
E Burke	12-19-01	Recorded by Kayla Dace	3-15-01

File: T2NC01_E04.21 Run Ended: Feb 28, 2001, 21:27:08 Signal G:17 A:36 T:38 C:20 Comment:
Sample: T2NC01_E04.21 Lane: 23 Base spacing 14.03 972 bases in 11551 scans Page 1 of 3



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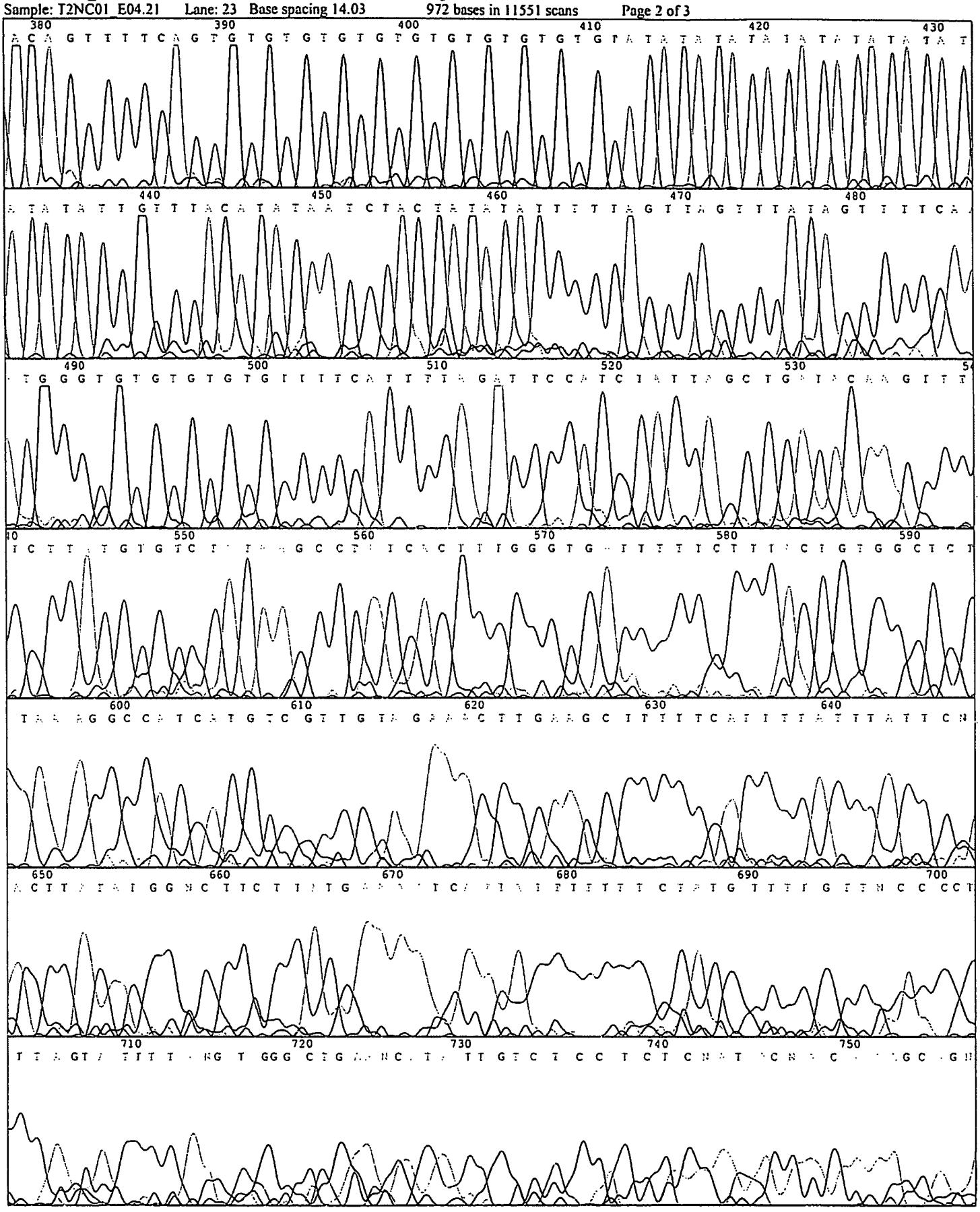
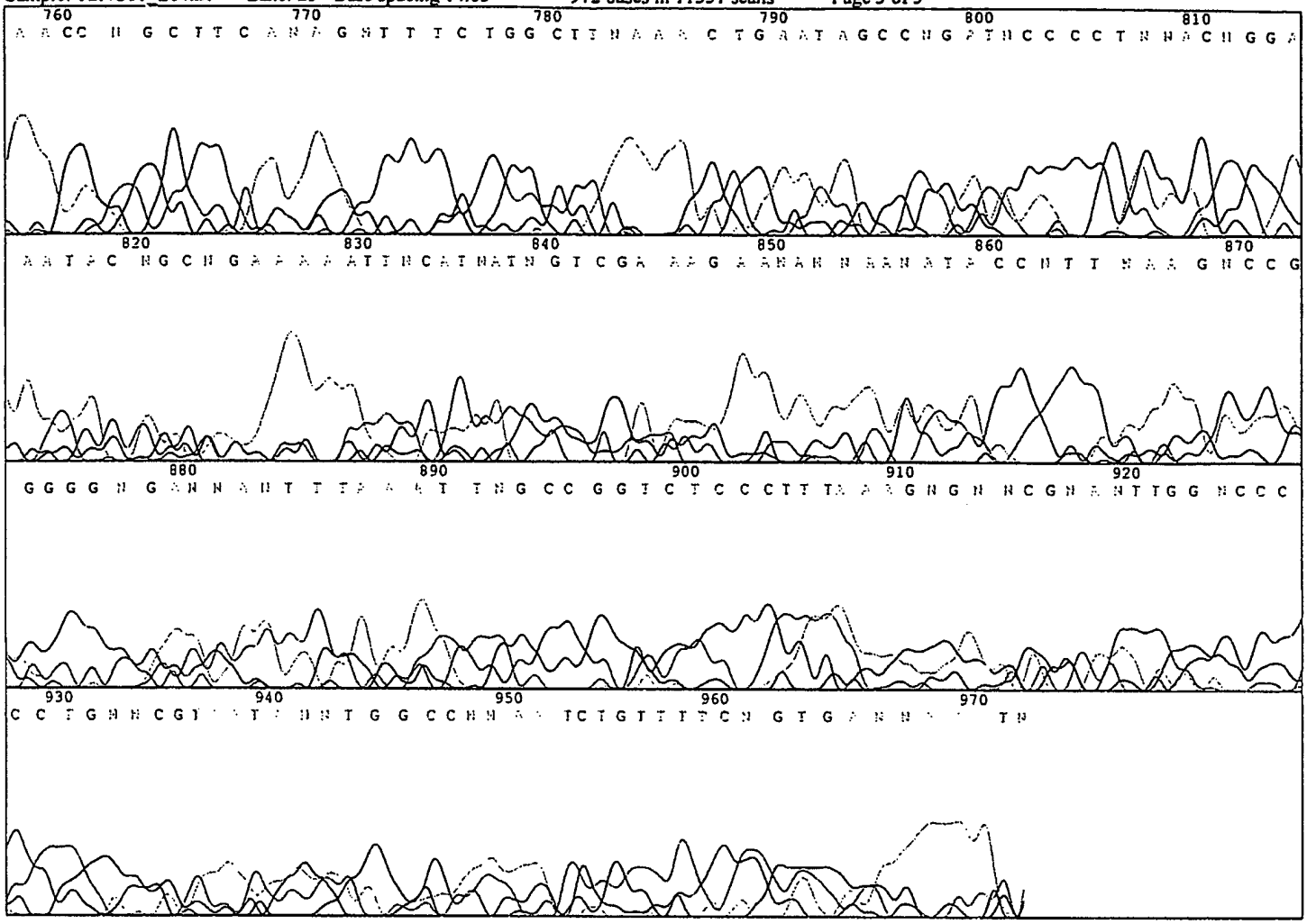
Sample: T2NC01_E04.21 Lane: 23 Base spacing 14.03 972 bases in 11551 scans Page 2 of 3



File: T2NC01_E04.21 Run Ended: Feb 28, 2001, 21:27:08 Signal G:17 A:36 T:38 C:20 Comment:
Sample: T2NC01_E04.21 Lane: 23 Base spacing 14.03 972 bases in 11551 scans Page 3 of 3



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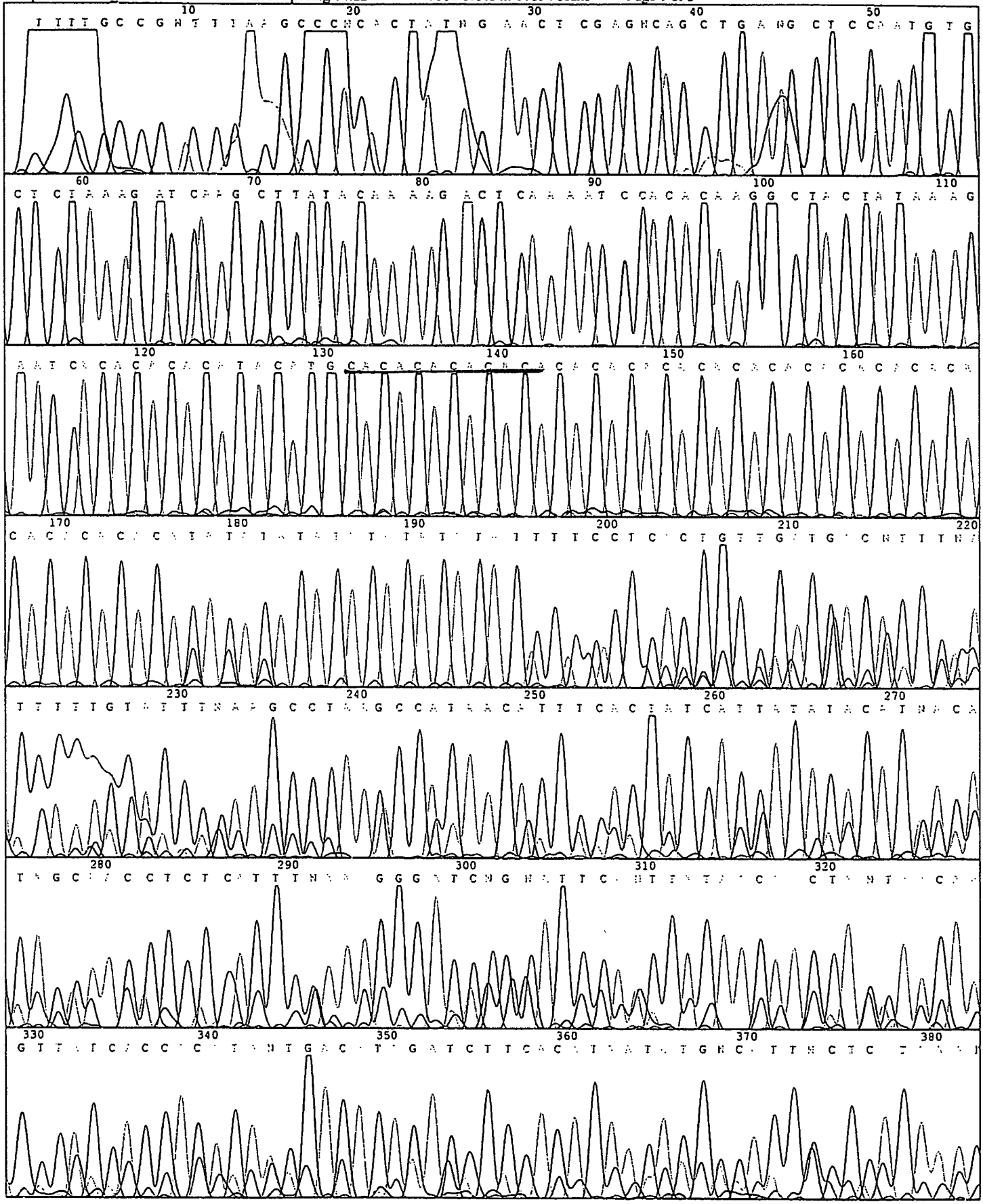
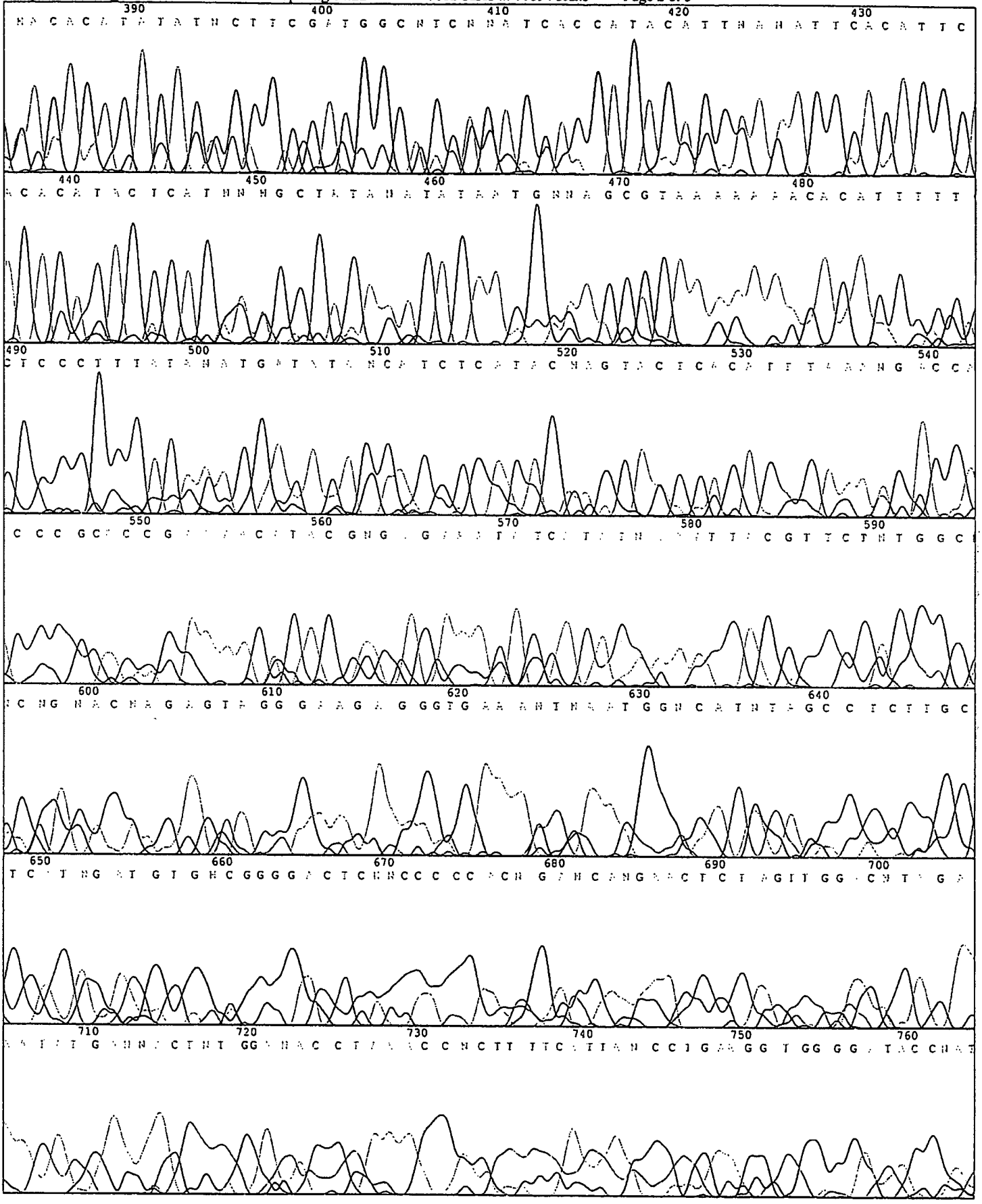
Sample: T2NC01_F09.21 Lane: 75 Base spacing 14.22 1002 bases in 11894 scans Page 1 of 3



File: T2NC01_F09.21 Run Ended: Feb 28, 2001, 21:27:08 Signal G:15 A:51 T:68 C:36 Comment:
Sample: T2NC01_F09.21 Lane: 75 Base spacing 14.22 1002 bases in 11894 scans Page 2 of 3



File: T2NC01_F09.21 Run Ended: Feb 28, 2001, 21:27:08 Signal G:15 A:51 T:68 C:36 Comment:
Sample: T2NC01_F09.21 Lane: 75 Base spacing 14.22 1002 bases in 11894 scans Page 3 of 3